

THE VEGETATIVE PROPAGATION OF DIFFICULT PLANTS

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With plates 228-231

INTRODUCTION

PROPAGATORS and gardeners have long been familiar with the fact that certain plants are readily propagated from cuttings, while others are not. The latter, which may be termed "difficult" or "resistant" plants, belong to many taxonomic orders, and there is no reason to associate their failure to form roots with any anatomical peculiarities. The discovery of the rôle of hormones in root initiation and the identification of the auxins as the substances primarily concerned (for literature see Went and Thimann, 1937) led of course to the supposition that failure to root was due to an insufficient supply of auxin. Hence the application of auxin to cuttings of such plants should induce root formation. In part this expectation was justified. Application of auxin in suitable concentration both hastens root development and increases the numbers of roots formed, on most plants which are capable of rooting at all. The list of plants for which data on the promotion of root formation by auxin treatment have been given is now very large (Chadwick, 1937; Cooper, 1935, 1938; Gočolašvili and Maximov, 1937; Hitchcock and Zimmerman, 1936; Laibach and Fischnich, 1935; Müller, 1935; Poesch, 1938; Pearse and Garner, 1937; Pearse, 1938; Traub, 1938; Went, 1934).

Nevertheless, it was soon found that many of the most resistant plants, which ordinarily are never propagated by cuttings, are not even induced to form roots by auxin treatment. So far as trees are concerned, these plants comprise three main groups: — (1) a majority of the conifers, (2) many forest hardwoods, (3) the apples and related rosaceous trees. There are a number of others. Evidently failure of these cuttings to root is not primarily due to insufficient auxin supply, and this is *a priori* reasonable, because we know that auxin is universally present at least in the spring and during the period of growth, so that if auxin supply were the controlling factor cuttings of "difficult" plants should at least root when taken at this time of year.

We have therefore considered it of importance to make a general

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study of the rooting of "difficult" plants, particularly since our present knowledge of auxin relations, and the unlimited availability of pure synthetic auxins, make it possible to control this one factor adequately. The study was made more urgent because the work of the Maria Moors Cabot Foundation involves the selection and breeding of conifers and forest trees, many of which fall into groups (1) and (2) of the above classification of "difficult" plants.

Much of our work has centered upon white pine (*Pinus Strobus*) but the principles brought to light have in each case been tested on other conifers and hardwoods. Data on the third group of trees will not be included in this first report.

Occasional reports of the rooting of cuttings of white pine and other *Pinus* species have appeared in the literature (see, for instance, Mirov, 1938, and literature there cited). Such reports are of little use for our purposes, however, firstly because quantitative data on the percentage of cuttings which rooted are rarely, if ever, given, and secondly because the exact conditions which brought about rooting on the particular occasion reported, as opposed to the numerous and widespread failures, are not investigated. In order to be of use for selection work, rooting must be under control and must be at least partially quantitative. As far as possible, therefore, we have attempted to define our conditions and to report all data quantitatively. Repetition of the experiments elsewhere should involve no difficulty.

In the course of the work a few trees have been found which are in fact readily rooted by auxin treatment alone. The most notable of these is the Canadian hemlock (*Tsuga canadensis*). *Picea pungens* behaves similarly. Successful rooting of white pine and others involves, however, other considerations.

The factors mainly studied have been:—

- (1) The age of the tree from which cuttings are taken. This is by far the most important single factor.
- (2) Optimal auxin treatment.
- (3) The relative rooting behavior of different parts of the plant.
- (4) Rooting medium and temperature, including treatment of the bases of the cuttings with hot water or permanganate.
- (5) Factors other than auxin, namely, sugar and vitamin B₁.

MATERIAL AND METHODS

All cuttings unless otherwise specified were of wood in its first year of growth, i.e. from 4 months up to one year old, or "one-year wood."

Cuttings of *Pinus Strobus*, *Tsuga canadensis* and its variety *pendula*, *Taxus baccata* and its variety *repandens*, *Picea Abies* and *Picea pungens* were obtained from trees of known age at the Arnold Arboretum. Those from young trees were obtained in part from the Harvard Forest Nursery (courtesy of Dr. Paul R. Gast) and in one case from a commercial grower. Cuttings of *Keteleeria Davidiana* Beiss. were from a tree about 10 years old at Cambridge. All were taken at the base of the one-year growth.

Hardwoods. Cuttings of *Fraxinus americana* were obtained from trees of known age at the Arnold Arboretum. Young trees of *Acer platanoides* as well as cuttings from old trees of *Populus nigra* var. *italica* and *Populus grandidentata* were collected in the vicinity of Cambridge. Young trees of *Quercus borealis* were obtained commercially. As far as possible all were taken with a "heel" at the base of the one-year wood.

Treatment. The cuttings were placed with their bases immersed 1-2 cm. deep in the auxin solution or water at room temperature for 24 hours, rinsed and placed in propagating boxes. Unless otherwise stated, the auxin used was indole-3-acetic acid (Eastman or Hofmann-La Roche). Sugar and other treatments are described below. The boxes were of cypress, fitted with a hinged glass cover which was generally kept nearly closed. During the summer the boxes were covered with two thicknesses of cheesecloth to reduce the light. The boxes were kept in the greenhouse with a minimum winter temperature of 60°F (15°C). The bottom was covered with coarse gravel to a depth of one inch, then by about 5 inches of sand, sand-peat mixture or pure peat. The mixture was found the most practical on the whole. Bottom heat, where used, was supplied by means of a G. E. unit no. 69×787. The medium was kept as moist as possible consistent with good aëration.

The experiments were carried out, in most cases, by dividing a group of several hundred cuttings into smaller groups each of which received a different treatment. For example, 300 cuttings might be divided into 4 groups of 75 for different auxin concentrations, and each of these subdivided into 3 groups of 25 to compare peat, sand and peat-sand mixture as rooting medium. Though the final number in any one group was thus small, the effect of any treatment can be determined from quite a large number of cuttings by adding together all those receiving that treatment in the different groups. This procedure, which must, of course, be applied with discretion, allows the effects of a number of variables to be surveyed. The important points can then be checked by separate experiments.

Since some 50,000 cuttings have been studied in small groups in this way it will not be possible to give the whole of the data in undigested form, but the principal conclusions and the evidence for them will be presented.

RESULTS

1. THE AGE OF THE PLANT

A large number of experiments with both soft and hard wood cuttings of *Pinus Strobus* (white pine) and *Quercus borealis* (red oak) taken from mature trees at different times of the year, treated with various concentrations of auxin up to 400 mg. per liter and kept in different rooting media, showed that in no case was there an appreciable percentage of rooting. The cuttings often survived for months, sometimes with development of buds or leaves, but eventually withered. It was not until attention was turned to cuttings from young trees that satisfactory rooting was attained.

It was then found that cuttings of white pine taken from trees two or three years old rooted rather quickly even without auxin treatment. With auxin the rooting was somewhat hastened and the percentage of cuttings rooting greatly increased. The results of a number of experiments on cuttings from trees of different ages are summarized in table 1.

It is clear that the percentage of rooting in water controls decreases steadily with increasing age. Cuttings from the older trees will not root appreciably whether treated with auxin or not. The rooting reported by Mirov (1938) from ten-year old trees must have either been in extremely low percentage*, or else his conditions were in some way more favorable than ours. Cuttings from the very young plants root readily. There is an intermediate age of about 3 years in which water controls root sparingly, but the percentage may be raised to 50 (or in occasional cases to 75 or 100) by treating with auxin. As will be shown in section 2, the optimum concentration of indole-acetic acid for 24-hour treatment of such young pines is usually 200 mg. per liter.

Plate 228, figure 1 shows the appearance of some of the cuttings. The roots are thick, few in number and grow laterally. All groups showed very high percentage of survival and gave excellent growth. In one case a short shoot, i.e. single fascicle of needles, from a 3-year old tree formed roots and started to develop a terminal bud.

This effect of age is evidently widespread. It is shown markedly by spruce, maple and oak and also probably by ash. Table 2 summarizes experiments with one species of each of these and Plate 228, figure 2

*No numerical data are given in his publication.

shows a sample of the *Picea Abies* cuttings. It is notable that with the 6-months old maples auxin actually reduced the rooting, probably because the concentration (100 mg. per liter) was too high for such young material (cf. the youngest pines in table 1). A number of other experiments with maple have been lost through infection. On the whole the maples so far studied have shown little increase in rooting from auxin treatment. In all the species shown in table 2, as well as with *Aesculus* and *Ginkgo*, with which a few trials have been made, rooting is far more prevalent when cuttings are taken from young than from old trees.

2. OPTIMAL AUXIN TREATMENT

In order to minimize variables, all cuttings have been treated at the base with aqueous solutions of auxin for 24 hours at room temperature. Sufficient leaf surface was always present to allow of reasonable transpiration. Where leaves were to be cut back this was never done before the auxin treatment. For each plant a series of concentrations was tested, using this one exposure time and treating all cuttings the same way. Controls were placed in water for the same period.

As mentioned above, some conifers were found to root readily even on cuttings taken from old trees (70 years or more). Results from six such species are given in table 3. The most striking result is the readiness of rooting of hemlock (*Tsuga canadensis*) on treatment with auxin, when untreated cuttings in no case formed a single root (see Plate 229, figure 1). Hemlock, at least when cuttings are taken in the fall, is thus a good example of a plant adequately supplied with all factors for rooting except auxin.

The extreme slowness of rooting on the whole, especially in the yews, is also remarkable. In both yew species, however, roots appeared earlier on auxin-treated cuttings than on the controls.

It is impossible to make any general deduction about optimal auxin concentration. Each species apparently has its own optimum. In many, auxin 400 mg. per liter is highly toxic and results in killing of the tissue from the base up to the level reached by the solution. While this may lead to decreased rooting it does not always do so. Thus the *Taxus baccata* cuttings in table 3 were killed up to 2 cm. or so above the base, but they nevertheless rooted vigorously above the killed zone. Good rooting above the killed zone is also shown by other species, notably *Tsuga* (Plate 229, figure 2) and *Picea* (Plate 229, figure 3). This fact may be of theoretical importance (see discussion). Another difficulty arises from the fact that the concentration actually optimum for root-formation may not be optimal in practice, because cuttings exposed to

the higher concentrations frequently show extreme inhibition of the buds. Thus although the blue spruce (*P. pungens*) rooted freely in auxin, bud development was practically prevented for some months in all but a few specimens. In *Populus*, also very marked bud inhibition was shown on cuttings treated with 100 mg. per liter.

The variability of the "optimum" is further shown in respect to root number. As is well known, cuttings of aspen (*Populus tremuloides*) and Lombardy poplar (*P. nigra* var. *italica*) root readily. Plate 230, figure 1 shows the numbers of roots produced in 3 weeks. All cuttings, including water controls, produced one or more roots in this time. The reduction in root number at high concentrations is of course due to killing of the basal tissue, which was preceded by much swelling and splitting of the bark caused by vigorous cell enlargement in the cortex. The interesting point of Plate 230, figure 1 is that while *P. nigra* var. *italica* shows a reduction in root number at 100 mg. per liter, *P. tremuloides* shows a very large increase at this concentration. Both sets of cuttings were of the same age, taken at about the same time of year, and treated identically.

The corresponding data for young trees of some of the more difficult species are shown in table 4. Points worth noting are: — (a) the high percentage rooting of the young spruces treated with relatively weak auxin; (b) the high percentage reached by the young oaks in 400 mg. per liter; these were basal parts of the trees, the wood being 2 to 3 years old, and showed little or no killing by this high concentration (see section 3); (c) the failure of maple cuttings to respond notably to auxin treatment; (d) the rather extensive rooting of the white pines, 3 years old.

These data confirm and extend those of table 1; the second set of 3-year pines in table 1 is in fact the mean of the separated figures for lateral and terminal shoots in table 4. The others listed are from separate large experiments. The overall percentage rooting in these large groups does not much exceed 50%, but in individual smaller groups it is often greater (see table 6).

Again these data do not allow of any statement as to the optimal auxin concentration. Taking all of our experiments on white pine together, however, the optimum is 200 mg. per liter. The statement of Went and Thimann (1937) that "the highest non-toxic concentration of indoleacetic acid, dissolved in water, will give the best results" remains as near the truth as any.

Other methods of applying auxin, as in lanoline paste or in fine dust with talc, are also under test on cuttings of these types and may be reported on later.

It has recently been reported by Cooper and Went (1938) that a second auxin treatment some time after the first may give good results. Our own experiments on such "retreatment" are not very numerous. In one experiment 3-year old white pine (taken in March) treated 24 hours with 100 mg. per liter auxin gave only 4% rooting; when the treatment was repeated 3 days later the final rooting was 9%. On another occasion the same concentration produced 15% rooting on 3-year old and 15% rooting on 4-year old white pine; when retreated 3 days later the final values were 25% and 18% respectively. Thus in each case there was a small increase in the percentage rooting. The time for rooting was about 10 weeks in each case. Continually repeated treatments, though inconvenient in practice, might perhaps be more effective.

From time to time statements have been made that indole-butyric or α -naphthalene-acetic acids are more effective for root-formation than indole-acetic. Such comparisons as we have made do not bear this out. The differences are not large and are usually in favor of indole-acetic acid. *Syringa vulgaris* (var. "de Louvain,") taken in April, treated with 50 mg. per liter of each auxin, gave after 5 weeks 14% rooting with indole-acetic, 3% with α -naphthalene-acetic and 0% and 8% in two experiments with indole-butyric. Similarly Concord grapes (cuttings of one-year wood taken in May) gave 87% in indole-acetic and 73% in indole-butyric acid; the average number of roots formed was 11.1 with indole-acetic and 7.6 with indole-butyric acid. The water controls gave 60% rooting with an average root number of 1.4. It is possible that the contrary statements reported elsewhere are due to the use of impure samples of the substances.

Another statement occasionally made is that the salts of the auxins are more effective than the free acids. In so far as growth promotion is concerned this has been disproved by D. Bonner (1937) and Thimann and Schneider (1938). However, the data of the latter authors showed that, at very high concentrations, the toxicity or damage effect was reduced by neutralizing the acids to form salts. Since such high concentrations are just those used in propagation, a comparison was made between the free acid and the salt with indole-acetic, using cuttings from 3-year old white pine (table 5). Each figure in the table is based on 50 to 70 cuttings. The differences are insignificant.

3. THE RELATIVE ROOTING ABILITY OF DIFFERENT PARTS OF THE PLANT

As shown in table 4, the lateral branches of *Pinus* consistently root better than the terminal shoots. The mortality among the lateral cut-

tings is also less. Table 6 shows in more detail the percentage rooting in water and in optimal auxin (either 100 or 200 mg. per liter indole-acetic acid) for 5 groups of lateral and terminal cuttings. Each figure is the average of 20 to 60 cuttings. In all groups but one the laterals give the higher rooting percentage in water controls, and in all 5 groups the higher percentage in auxin, 100% being reached in one case. The mortality among water controls is also significant. After a month 32% of all terminals treated with water had died, but only 3% of all laterals. Other experiments gave comparable results. One group of pine cuttings, taken in July, which rooted very poorly, gave only 0.4% rooting in terminals, as compared with 7.9% in laterals. Norway spruce (also included in table 6) shows the same phenomenon. The average behavior of a still larger number of cuttings from 3-year old white pines and Norway spruces is summarised in Plate 230, figure 2.

In view of the occasional records of rooting by layering of lateral branches under natural conditions it is possible that the ability of laterals to form roots (of course only to a very small extent, and not in *Pinus* itself) persists up to a considerable age.

It is interesting to note that the plagiotropic habit of growth is retained in lateral branches of *Picea* after they have rooted. As Plate 230, figure 3 shows, the new growth which develops after rooting is always at an angle to the vertical, while that of terminal shoots is erect. Of a total of 53 rooted laterals which were potted for observation, every one grew at an angle in this way, while all of the 18 rooted terminals which were potted grew vertically. This appears to be in conflict with observations such as those of Errera (1905) that when *Picea Abies* is decapitated one of the laterals grows vertically, for in that case the influence of the tip would only have to cease in order for the lateral growth to be changed. Our observations show that whatever influence has caused the plagiotropism of these laterals is by no means lost when they are isolated from the tree. It may, of course, be that in subsequent years these cuttings (which have been planted out) will change their habit. In *Pinus* this phenomenon is not shown, and rooted laterals seem to grow just as vertically as the terminals (Plate 231, figure 1).

Another difference in the rooting of different parts was found with oak and maple. Here, in young trees, the lateral shoots are short and not very vigorous. The apical part of the plant, comprising the last year's growth, roots rather poorly, and its rooting is not promoted appreciably by auxin treatment. The basal part, on the other hand, roots well and responds strongly to auxin. As table 7 shows, the bases of *Quercus borealis* can withstand 400 mg. auxin per liter and give very high per-

centage rooting therein; even in water controls there is moderate rooting. Some of the rooted cuttings are shown in Plate 231, figure 2. The data for *Acer* show the same effect. Here the numbers in each auxin group were too small for significant results, so that only the combined results are given. The reason for this behavior is not yet known (see discussion), but its practical importance for propagation when young trees are used is evident. The difference between apex and base has not yet been studied in conifers.

4. MEDIUM AND TEMPERATURE CONDITIONS

A few comparisons of different rooting media are shown in table 8. On the whole either peat or the 2:1 mixture of sand and peat give slightly higher percentages of rooting than sand alone. On account of its high water content pure peat is apt to become very cold, and unless bottom heat is used this is a drawback. There was also (in *Pinus*) a higher percentage of rotting when the rooted cuttings were left in the peat than in the other two media. Since the differences are in no case large the mixture was mainly used.

The use of bottom heat for *Pinus* cuttings is definitely undesirable. Rooting was slower and of lower percentage in heated than in unheated boxes. The unheated boxes had a mean winter temperature close to 18°, the heated were maintained at 24°. In both cases somewhat higher temperatures (up to 27°) were reached in summer, even though the boxes were kept covered with cloth. Comparisons were not made on cuttings other than pine, since in many cases a temperature around 24° is known to be advantageous.

Treating the cuttings with permanganate (cf. Curtis, 1918) is also undesirable where auxin is to be applied basally. In our experiments the bases of the cuttings after placing in 0.1% KMnO_4 were thoroughly rinsed, but the data indicate that traces may have remained sufficient to inactivate part of the auxin afterwards applied. Indole-acetic and butyric acids are very sensitive to oxidation. The results for the two conifers in table 9 show only a reduction in rooting by permanganate treatment. In other experiments white pine also showed a small reduction. With poplar the effect of the treatment on the number of roots was studied, but though there was a slight increase in root number on the controls, the response to the auxin was decreased (table 9). It is notable, however, that those exposed to the highest auxin concentration were partially protected from killing by the permanganate. This may be due to partial auxin inactivation or perhaps to plugging of the vessels by the precipitate of manganese hydroxide and hence reduction in the

amount of auxin taken up. When auxin is applied at the apex, as in the standard pea-rooting test (Went, 1934; Thimann and Went, 1934) or with lanoline (Laibach, 1933, Laibach and Fischnich, 1935), permanganate inactivation of auxin probably does not occur because the auxin is already within the cells when it reaches the base.

Another frequently used procedure for pines and other resinous plants is to soak the bases of the cuttings in hot water to remove the resin. In a series of comparisons in which cuttings from 3-year old white pine were immersed in hot water at from 40° to as high as 90° for times between 2 minutes and 2 hours no beneficial effect was observed. The percentage was never above, and sometimes below, that of cold water-treated controls. Thus in one set of experiments the mean of all auxin concentrations for hot water treated cuttings was 14%, that of cold water-treated controls 17%. In another set the values were 15% and 24% respectively. In view of the fact that resin was certainly exuded from the bases of the cuttings, there seems no reason to believe that this in itself has any inhibitory effect on rooting. Usually the resin came away readily enough on wiping or on placing in the propagating boxes. Hot water treatment has not been tested on other conifers.

5. SUBSTANCES OTHER THAN AUXIN

The action of auxin is to cause the initiation of roots. The root initials thus formed must then grow out, and this growth requires nutritive materials. Probably carbohydrate is the most important single factor for such growth. In experiments with etiolated plants, rooting is definitely dependent on the supply of sugar (Bouillenne and Went, 1933; see Went and Thimann, page 194, figure 62). Plants in the light, with leaves, might be expected to have an adequate carbohydrate supply, but our experiments show that this is not necessarily true. Table 10 gives representative data from ash and white pine cuttings, which were immersed in the auxin solution (or in water) for 24 hours as usual and then placed in 5% sucrose for 3 days, rinsed and transferred to the propagating boxes. All figures except the last pair represent percentage rooting. The last pair of figures shows that the mortality among the cuttings is reduced by sugar treatment. This effect became marked early in the experiment, most of the non-sugar cuttings which had not rooted dying off within 6 weeks, while in the sugar-treated cuttings a large proportion of the unrooted cuttings still lived. When it is remembered that one of the major problems of propagation is maintenance of the unrooted cuttings, the value of such sugar treatment is clear. The increase in percentage rooting may be due, at least in part,

to this action of sugar on maintenance. The young ash cuttings in table 10 gave poor rooting and the data are not significant.

Another substance known to be essential for growth of roots is vitamin B₁ or aneurin. Went, Bonner and Warner (1938) have increased the rooting of camellias and other plants considerably by treatment with this substance. The effect is presumably not exerted on root initiation itself but only on subsequent growth of the roots. Our own experiments on vitamin B₁, while not very numerous, have not shown very marked effects. When it was first found that cuttings from old pines did not root after treatment with auxin, the action of vitamin B₁ was tested. Of a large group of cuttings taken in September from a tree 65 years old, a number were treated for 24 hours with 1 mg. per liter of vitamin B₁ following auxin treatment, but none rooted. In another group the cuttings were given the same concentration of vitamin B₁ two months after auxin treatment, and also given sugar. One of the cuttings later rooted. With *Fraxinus*, a group of cuttings which had not rooted one month after auxin treatment were given the vitamin, and 7 weeks later 10% had rooted, while the control group not given the vitamin but having received the same auxin treatment gave no rooting. Another group of *Fraxinus* which were given sugar as well as auxin gave 2% rooting when receiving no vitamin and 5% when given 1 mg. per liter for 24 hours one month after the auxin treatment.

The results are thus favorable to the vitamin treatment, though the effects are small, and it may be deduced that, as with carbohydrate, the amount of vitamin B₁ present or synthesized in the cutting is probably not optimal. However, it is also evident that neither the vitamin nor carbohydrate is the factor which is missing from old trees.

A few experiments have also been made with a biotin preparation (from egg yolk), biotin being also known to be of importance in rooting (see Went and Thimann, page 196). The data are insufficient for report here, but it is of interest that one cutting from old pines rooted when the auxin treatment was followed by biotin.

More systematic trials with these substances are of course in progress.

DISCUSSION

Without doubt the main fact of importance which the experiments have shown is that the tendency to form roots is controlled not by the age of the cutting, but by the age of the tree from which the cutting is taken. This is, of course, in some ways an old observation, since as long ago as 1913 Goebel pointed out that cypress cuttings taken from juvenile plants root more readily than those from mature trees. The

literature contains several scattered reports of the same type, and one very important systematic study by Gardner (1929). Of 21 trees studied, including seven conifers, all but two species showed very much better rooting when cuttings were taken from one-year seedlings than from old trees. Where two- and three-year old trees were studied the percentage rooting decreased with increasing age. The recent study of Stoutemyer (1937) confirms the findings of Gardner in that the tops of one-year apple seedlings seldom failed to root. Stoutemyer further shows that ease of rooting in these young plants is associated with juvenility of leaf form. Thus adventitious shoots from the root had juvenile foliage and rooted readily, while normal twigs or adventitious shoots from the trunk had adult foliage and failed to root. Auxin treatments were not studied.

From a practical point of view, as Gardner points out, it is of little use to make cuttings from one-year old plants since almost the whole plant is used. By taking advantage of the fact that up to 3 or 4 years old the ability to root is still present if auxin is supplied, our experiments show that white pine and many other "difficult" plants can be readily rooted and may be propagated vegetatively in a practical way. No doubt the responsive period can be extended another year or two and this will be further studied. In view of the representative nature of the trees we have used, including both gymnosperms and angiosperms, the above is doubtless a general principle, and for selection work it might be said that the problem of the rooting of "difficult" plants is partially solved.

The explanation of this ease of rooting is, however, by no means easy, and the theoretical problems are not solved but actually increased. It is difficult to associate the ease of rooting (as did Stoutemyer with *Malus*) with a stage of "juvenility," for two reasons; — firstly because many of the trees used do not show any definite juvenile characters, either of leaf or growth habit, at the age of 3–4 years,* and secondly because the effect is evidently quantitative rather than qualitative, the ease of rooting falling off steadily with increasing age. On the contrary, it is evident that there is a *physiological juvenility*, which is quantitative in character, and which controls the ease of rooting in presence of sufficient auxin. This physiological juvenility may or may not produce qualitative morphological differences, according to the species.

Further, along with the age effect must also be considered the differences within the plant, lateral shoots rooting better than terminal, and

*But note the difference between the foliage of 3-year old and 65-year old *Pinus Strobus* in Plate 231, figure 1..

basal pieces better than apical. In all these cases, not only is the rooting better in water controls but the increase due to auxin is also greater (see Plate 230, figure 2). Hence the differences cannot be due to differences in auxin content of the cuttings. The experiments with substances other than auxin, while admittedly very scanty, show clearly that these are not the main factors which are missing from old trees or from poorly-rooting cuttings in general. Some *internal factor* of an unknown kind is evidently concerned. It would be easy to postulate a special substance, whose formation decreases with age, and whose distribution within the plant is responsible for the differences in rooting of the different parts such as the "rhizocaline" postulated by Went (1934, 1938) and Cooper (1938). In this connection it is important to note that in *Taxus*, *Pinus*, *Tsuga* and *Picea*, very high auxin concentrations, sufficient to kill the base of the cutting, by no means prevent root formation, but roots are formed in good numbers above the killed zone. According to the theory of Went (see especially Cooper, 1938) the auxin should draw the "rhizocaline" down to the base of the cutting, and if the base is then cut off or allowed to rot away all the rhizocaline would be lost along with it. Our experiments, therefore, while by no means conclusive, do not support this view of auxin action. The data of Pearse (1938) on *Salix* also do not agree with those of Cooper on *Citrus*. Apart from the "rhizocaline" theory, however, it remains very probable that the *internal factor* is a special substance or group of substances.

It is, however, also possible that the differences in rooting ability between old and young, or between terminal, lateral and basal parts, are morphological in nature. Specifically, they might be due to the absence in the poorly-rooting cuttings of certain types of cell which are those responsible for root initiation. In view of the recent finding of Dorn (1938) that roots are apparently initiated in different cell layers in different plants this does not seem very probable but must still be considered. An anatomical study of rooted cuttings taken from a series of ages would throw light on this problem. As yet the evidence does not allow of any decision between these two possibilities.

SUMMARY

1. A study has been made of the vegetative propagation of a number of coniferous and deciduous trees which are known to form roots from cuttings only with extreme difficulty.
2. The most important single factor in rooting these "difficult" trees is the age of the tree from which cuttings are taken. The ease with which roots are formed (on cuttings of one-year wood) falls off steadily with

increasing age of the tree. This applies both in the presence and in the absence of auxin treatment.

3. When cuttings are taken from trees 3 to 4 years old, and treated with the optimal concentration of indole-acetic acid or other auxin, excellent rooting is obtained with white pine (*Pinus Strobus*), Norway spruce (*Picea Abies*), red oak (*Quercus borealis*), Norway maple (*Acer platanoides*) and to a less extent with American ash (*Fraxinus americana*).
4. The optimum concentration of auxin depends upon the material. Using aqueous solutions applied to the base for 24 hours, it varies between 25 and 400 mg. per liter.
5. In *Pinus* and *Picea*, lateral shoots root more readily than terminal, and in *Quercus* and *Acer*, the bases of the young plants root more readily than the apices.
6. Rooted lateral branches of *Picea Abies* retain their plagiotropic habit of growth for at least one year, while those of *Pinus* apparently do not.
7. Exposure of the bases of the cuttings to permanganate or hot water before auxin treatment did not promote rooting in any of the species.
8. Application to the *Pinus* cuttings of sugar solution, following treatment with auxin, increased the percentage rooting and decreased the mortality rate.
9. A second treatment with auxin some days after the first, or a treatment with vitamin B₁, may have a small beneficial effect.
10. In some conifers, notably *Tsuga canadensis* and *Picea pungens*, cuttings from old trees root exceedingly well if treated with auxin but not at all in absence of auxin.
11. Basal treatment with high auxin concentrations frequently inhibits development of the buds on cuttings, sometimes for several weeks. In other cases it may cause killing of the base, but this does not necessarily interfere with good root formation.
12. Root formation in presence of optimal auxin is apparently controlled by an internal factor whose amount and distribution varies with the location of the cutting on the tree and with the age of the tree.

TABLE 1.

EFFECT OF AGE OF THE TREE ON THE ROOTING OF WHITE PINE CUTTINGS.

Age of tree in years	Percentage of cuttings rooting in auxin of concentration:				Approximate time for rooting in weeks.
	0	100	200	400 mg. per liter	
½ to 1	31	20	—	—	6
2	18	46	—	—	7
3	1	10	31	50	11
3 (another experiment)	18	36	37	27	8
10	0	0	—	—	
65	0	0	0*	0*	15 to 21

* Occasional rooting but less than 1%.

TABLE 2.

EFFECT OF AGE OF THE TREE ON THE ROOTING OF SPRUCE, MAPLE,
ASH AND OAK CUTTINGS.All data the mean percentage rooted, terminal and lateral cuttings being
combined. Auxin concentration 100-200 mg. per liter.

Species	Age of tree from which cuttings were taken (years).							
	½		2		3 and 4		60 or over	
	Water	Auxin	Water	Auxin	Water	Auxin	Water	Auxin
<i>Picea Abies</i>	—	—	—	—	35	78	0	0
<i>Acer platanoides</i>	67	7	40	60	31*	30*	—†	—†
<i>Fraxinus americana</i>	—	—	—	—	0	7	0	1
<i>Quercus borealis</i>	—	—	—	—	9.5	18	0	0

* Combined data from 3, 4 and 5 years.

† Occasional rooting but less than 1%.

TABLE 3.

EFFECT OF AUXIN TREATMENT ON THE ROOTING OF CUTTINGS FROM
OLD TREES OF VARIOUS CONIFERS.

All data the mean percentage rooted, terminal and lateral
cuttings being combined.

Species	Month in which the cuttings were taken.	Auxin concentration in mg. per liter.					Approximate time for rooting in weeks.
		0	50	100	200	400	
<i>Taxus baccata</i> <i>repandens</i>	October	40	—	95	—	100	24
<i>Taxus cuspidata</i>	June	0	—	—	29	—	10
<i>Tsuga canadensis</i>	October	0	—	8	—	79	10
<i>Tsuga canadensis</i>	October	0	—	50	—	100	21
<i>Tsuga canadensis</i> <i>pendula</i>	December	0	63	54	—	—	8 to 11
<i>Picea pungens</i> <i>Moerheimii</i>	April	0	—	80	60	14	8
<i>Keteleeria Davidiana</i>	December	0	—	100	—	—	7

TABLE 4.

EFFECT OF AUXIN TREATMENT ON THE ROOTING OF CUTTINGS
FROM YOUNG TREES.

All data the mean percentage rooted.

Species and age	Month in which the cuttings were taken.	Auxin concentration in mg. per liter.				
		0	25	100	200	400
<i>Pinus Strobus</i> 3 years	October	38	—	30	—	55
<i>Pinus Strobus</i> 3 years	February	7	—	20	29	23
<i>Pinus Strobus</i> 3 years (laterals only) (terminals only)	March	27	—	48	51	33
		9	—	24	23	20
<i>Picea Abies</i> 3 years	April	35	72	78	—	78
<i>Fraxinus americana</i> 4 years	March	0	—	7	5	0
" " " "	"	0	—	25	—	—
<i>Acer platanoides</i> 2 to 5 years	June	33	—	44	32	14
<i>Quercus borealis</i> 4 years (basal parts only)	February	22	—	30	38	82

TABLE 5.

EFFECT OF NEUTRALIZATION OF INDOLE-ACETIC ACID SOLUTION.

Pinus Strobus, 3 years old; lateral and terminal combined. Cuttings taken in February. All data the mean percentage rooted in 10 weeks.

	Auxin concentration in mg. per liter.			
	0	100	200	400
Free acid	} 5 {	20	10	12
Potassium salt		12	18	10

TABLE 6.

COMPARATIVE PERCENTAGE ROOTING OF TERMINAL AND LATERAL SHOOTS.

Percentage rooting in 11 weeks.

Treated with water		Treated with auxin	
Terminal	Lateral	Terminal	Lateral
<i>Pinus Strobus</i> , 3 years old; taken in February			
7	25	28	43
3	30	15	68
19	25	70	77
23	47	33	100
11	9	20	34
<i>Picea Abies</i> , 3 years old; taken in April			
20	41	56*	87*

* Mean of 25, 100 and 400 mg. per liter auxin.

TABLE 7.

COMPARATIVE PERCENTAGE ROOTING OF DIFFERENT PARTS OF THE PLANT.

	Auxin concentration in mg. per liter.				Mean of all treated with auxin.
	0	100	200	400	
<i>Quercus borealis</i> , 4 years old; taken in February					
Terminal shoots	8	3	9	2	5
Lateral shoots	0	0	8	0	3
Basal parts (cf. table 4)	22	30	38	82	55
<i>Acer platanoides</i> , 4 and 5 years old; taken in June					
Terminal shoots	0	—	—	—	18
Basal parts	—	—	—	—	50

TABLE 8.

PERCENTAGE ROOTING IN THREE MEDIA.

Material	Treatment	Peat	Peat and Sand	Sand
<i>Pinus Strobus</i> laterals	Auxin 100 to 400 mg. per liter	36	50	34
<i>Taxus baccata</i> all parts	Auxin 100 mg. per liter	87	—	73
<i>Quercus borealis</i> all parts	Mean of all	—	12	9
<i>Quercus borealis</i> basal parts	Auxin 100 mg. per liter	—	30	22

TABLE 9.

EFFECT OF THE BASAL APPLICATION OF PERMANGANATE
PREVIOUS TO AUXIN TREATMENT.

Species and month	Pretreatment	Auxin concentration in mg. per liter			
		0	25	100	400
<i>Tsuga canadensis</i>					
September	Water	0	—	50	100
(% rooting)	KMnO ₄	0	—	0	75
<i>Picea Abies</i>					
October	Water	63	78	78	89
(% rooting)	KMnO ₄	22	67	55	67
<i>Populus tremuloides</i>					
October	Water	4	18	39	0*
(No. of roots per cutting)	KMnO ₄	8	13	25	9

* Killed.

TABLE 10.

EFFECT OF SUGAR TREATMENT ON PERCENTAGE ROOTING AND ON SURVIVAL.

Material	First treated for 24 hours with:	Afterwards treated for 3 days with:	
		SUGAR	WATER
<i>Fraxinus americana</i> 70 years	Auxin 100 to 200 mg. per liter	11	0
<i>Fraxinus americana</i> 4 years	Auxin 100 to 200 mg. per liter	2	3
<i>Pinus Strobus</i> 3 to 4 years	Water	14	8
" "	Auxin 100 mg. per liter	17	5
" "	Auxin 100 mg. per liter retreated	22	6
<i>Pinus Strobus</i>	All auxin treatments Percentage still living after 10 weeks	36	15

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DESCRIPTION OF PLATES

PLATE 228

- Fig. 1. *Pinus Strobus*. From 3-year old trees. Left to right, 400, 100, 0 mg. auxin per liter. After 10 weeks.
- Fig. 2. *Picea Abies*. From 3-year old trees. Left to right, 400, 100, 25, 0 mg. auxin per liter. After 7 weeks.

PLATE 229

- Fig. 1. *Tsuga canadensis*. From tree about 40 years old. Left to right, 400, 100, 0 mg. auxin per liter.
 - Fig. 2. *Tsuga canadensis* var. *pendula*. From tree about 50 years old. Left to right, 100, 50, 0 mg. auxin per liter.
 - Fig. 3. *Picea pungens*. From tree about 60 years old. Left to right, 400, 200, 100, 0 mg. auxin per liter.
- All three after 9-10 weeks.

PLATE 230

- Fig. 1. Average number of roots per cutting formed in 3 weeks on two species of *Populus* treated with water and auxin.
- Fig. 2. Comparison of the percentage rooting of lateral and terminal shoots from 3-year old trees of *Pinus Strobus* and *Picea Abies*. The white columns represent the percentage rooting of the water controls, the black columns the additional percentage rooting resulting from optimal auxin treatment.
- Fig. 3. *Picea Abies*. From 3-year old trees. Left, lateral; right, terminal. Photographed 3 months after rooting, during which the new growth above the arrow has taken place.

PLATE 231

- Fig. 1. *Pinus Strobus*. Left to right, two laterals from 3-year old trees, two terminals from 3-year old trees, lateral from 65-year old tree. Photographed 6 months after rooting. Note vertical growth of laterals, also the difference in foliage between 3-year and 65-year trees.
- Fig. 2. *Quercus borealis*. Basal cuttings from 4-year old trees. Left to right, 400, 200, 100, 0 mg. auxin per liter.

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